Affinity", and U.S. Patent Application Number 60/252,617, entitled "Methods and Computer Software Products for Selection Nucleic Acid Probes Using Dynamic Programming", filed concurrently herewith. Both applications are incorporated herein by reference for all purposes.

A1

Please replace the paragraph on page 16, lines 9-16 with the following:

A2

In one aspect of the invention, a physical model that is based on the thermodynamic properties of the sequence is used to predict the array-based hybridization intensities of the sequence. Hybridization propensities may be described by energetic parameters derived from the probe sequence, and variations in hybridization and chip manufacturing conditions will result in changes in these parameters that can be detected and corrected. U.S. Patent Application Number 09/721,042, filed concurrently herewith and incorporated herein by reference, discloses methods for predicting nucleic acid hybridization affinity.

Please replace the paragraph on page 17, lines 17-21 with the following:

A3

There are a number of ways to establish the relationship between the sequence and ΔG. In preferred embodiments, one model (equation 2), shown in U.S. Application Serial Number 09/721,042, previously incorporated by reference is shown below:

$$\Delta G_{seq} = \sum_{i=1}^{3N} P_i S_i \qquad \text{[Equation 2]}$$

Please replace the paragraph on page 19, lines 1-5 with the following:

$$I = C_0 [P \bullet T] \qquad \text{[Equation 4]}$$

$$[P \bullet T] = K_s[P][T] = e^{-\Delta G/RT}[P][T] \qquad \text{[Equation 5]}$$

$$\text{LnI} = -\Delta G/RT + \text{Ln}\{C_0[P][T]\} \qquad \text{[Equation 6]}$$

$$LnI = C_1 \sum_{i=1}^{3N} P_i S_i + C_2, \text{ where } C_2 =$$

$$\text{Ln}\{C_0[P][T]\} \text{ and } C_1 = -1/RT \qquad \text{[Equation 7]}$$

or

$$LnI = \sum_{i=1}^{3N} C_1 P_i S_i + C_2 = \sum_{i=1}^{3N} W_i S_i + C_2 \qquad \text{[Equation 8]}$$

Please replace the paragraph on page 19, lines 8-10 with the following:

where $W_i = C_1 P_i$. The following is a linear regression model for probes of N bases in length using a training data set that contains intensity values of M probes.

$$Ln(I_1) = W_1 S_{11} + W_2 S_{21} + \ldots W_{3N} S_{3N1}$$
$$Ln(I_2) = W_1 S_{12} + W_2 S_{22} + \ldots W_{3N} S_{3N2}$$

.

.

.

$$Ln(I_M) = W_1 S_{11} + W_2 S_{12} + \ldots W_{3N} S_{3N1}$$

Please replace the paragraph on page 19, lines 11-16 with the following:

Hybridization intensities (relative to a reference base, such as an A) for each type of base at each position in the probe sequence may be predicted. Multiple linear regression analysis is well known in the art. See, for example, the electronic statistic book, Statsoft Inc; Darlington, R. B. (1990). Regression and linear models. New York: McGraw-Hill, both incorporated by reference for all purposes. Computer software packages, such as SAS, SPSS, and MatLib 5.3

Please replace the paragraph on page 21, lines 8-14 with the following:

where W_d is the weight for sequence based probe affinity; W_{PF} is the weight for probe formation and W_{PP} is the weight for probe dimerization. Any methods that are capable of predicting probe folding and/or probe dimerization are suitable for at least some embodiments of the invention for predicting the hybridization intensity in at least some embodiments of the invention. In a particularly preferred embodiment, Oligowalk (available at the University of Rochester's website) may be used to predict probe folding.

Please replace the paragraph on page 23, lines 5-19 with the following:

Figure 10 shows a computer-implemented process for selecting probe sequences from a pool of candidate probes. In this particular embodiment, the sequences of a pool of candidate oligonucleotide probes are processed by a quality predictor (101). Throughout this application, the term probe may refer to the sequence of a probe. The pool of candidate oligonucleotide probes may be all possible probes against a particular target or targets. Typically, oligonucleotide probes are at least 10, 15, 20, 25 and 30 bases in length.

Polynucleotide probes can be more than 10, 20, 25, 30, 100, 200, 500, 1000, or 5000 bases in length. Figure 11 illustrates a complete pool of candidate oligonucleotide probes (unfilled rectangular boxes) against a target (black rectangular box). Each of the probes is designed to be complementary to the target sequence. In this particular embodiment, the oligonucleotides are 25mers. The first probe is complementary to bases 1-25 (from the 5' end) of the target sequence. The second probe is complementary to bases 2-26 and so on. While a complete pool is often desirable, it is not necessary to have a complete pool for at least some embodiments of the invention. In some cases, filters may be used to remove some of the probes from the pool.

Please replace the paragraph on page 24, lines 4-8 with the following:

The quality predictor is a software module that calculates quality scores (the term score refers to any qualitative and quantitative values with regard to desired properties of a probe) for probes based upon the sequences of probes. In some embodiments, the quality score may include predicted values such as perfect match intensity, mismatch intensity and/or slope.

Please replace the paragraph on page 24, lines 17-21 with the following:

In preferred embodiments, the goal of probe selection step is to find the best probes to represent a sequence. The probe selection software module takes a set of probes and a set of quality measures for each probe. It then implements an optimization algorithm to find the best n probes, spread out across the gene. Methods for probe selection using optimization

algorithm is described in U.S. Application Number 09/745,965, filed concurrently herewith and incorporated herein by reference in its entirety for all purposes.

Please replace the paragraph on page 25, lines 3-16 with the following:

FIG. 12 shows another embodiment of the computer implemented probe selection process of the invention, target sequences are inputted to a candidate probe generator (121) which produce either all possible probes of certain length or a subset of the all possible probes. The candidate probe sequences are fed to the quality score predictor (122) for calculating quality measures (scores, e.g., perfect match intensity, mismatch intensity and/or slope). The candidate probe sequences are also fed to a 3' bias score predictor (123) to obtain 3' bias scores that indicates the distance of probe sequence from the 3' end of target sequence. Since the current target preparation method is 3' biased, it is important to select probes that fall into range where its target will be made. The probe sequences may optionally be inputted into a cross hybridization score predictor (124) to calculate cross hybridization scores. The quality scores, 3' bias scores and/or cross hybridization score are combined by a probe score calculator module (125) to produce a unified score.

Please replace the paragraph on page 26, lines 7-13 with the following:

The multiple probe FASTA sequence file is also inputted into a cross hybridization predictor (136) to predict a cross hybridization score. The cross hybridization score predictor is based upon models (such as multiple linear regression models) derived from experiment data (1311). In some embodiments, cross hybridization may also be evaluated by pruning

probe sequences against a human genome data base (1312) which may be residing locally, in a local area network or in a remote site such as the Genbank.

Please replace the paragraph on page 32, lines 8-15 with the following:

Figure 14 shows the overall process of the experiments. Yeast was used as a model system for this experiment because the yeast genome had been sequenced. Arrays containing nucleic acid probes complementary to yeast genes are commercially available from Affymetrix (Santa Clara, California). Genes were selected to cover sequence complexity such as GC content, secondary structure, Motif and gene clusters. Twenty probe pairs (perfect match and mismatch probes) were selected to cover entire sequence of one of the 112 selected yeast genes. The probes are synthesized in situ on glass substrate using photo-directed synthesis

REMARKS

Election/Restrictions

Applicants wish to thank the Examiner for vacating the restriction set forth in Paper No. 3 and allowing all pending claims to proceed to examination.

Objection to specification is obviated

The specification has been amended to address the Examiner's objections and typographical errors. Specifically, embedded hyperlinks and browser-executable code on pages 19, 21 and 26 have been deleted as have attorney docket numbers on pages 2, 16, 17 and 24. No new matter is presented by the amendments.